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SPECIFICATION

irrelevant isotype-matched control MAb G3-519. The Y-axis represents the % hemolysis inhibition. The X-axis represents the concentration of factor D.

Fig. 6 shows the inhibition of factor-D dependent EAC3b cell lysis by MAb 166-32. The alternative C3 convertase was assembled on EAC3b cells by incubation with factor B, factor P and factor D. Different concentrations of MAb 166-32 were added to the incubation buffer to inhibit the activity of factor D. The line marked with filled squares represents MAb 166-32. The line marked with filled circles represents MAb G3-519. The Y-axis represents the % hemolysis inhibition. The X-axis represents the concentration of the MAbs.

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Fig. 37 shows that MAb 166-32 (closed squares) inhibits the production of platelet thrombospondin in the extracorporeal circuits as compared to the negative control MAb G3-519 (open squares). The number of paired circuits is 5. Mean ± S.E.M.; $P = 0.016$ (*), $P = 0.003$ (**) and $P = 0.0001$ (***) by two-way ANOVA.

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GVB/Mg-EGTA) in a microtube and incubated for 15 minutes at room temperature. The blank contained no antibody; only the plain medium and the serum. After incubation, 50 µl of washed zymosan suspension were added to each tube for incubation for 30 minutes at 37°C. The microtubes were then centrifuged at 2000 x g for 5 minutes, the supernatants were collected and mixed with equal volume of Specimen Stabilizing Solution (Quidel, San Diego, CA). The samples were frozen at -25°C until being assayed. The concentration of C3a and sC5b-9 in the samples were measured by quantitative ELISA kits (Quidel) according to the procedures provided by the manufacturer.

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In order to examine whether the monovalent form of MAb 166-32 is as effective in inhibiting the alternative complement pathway as the parental bivalent MAb166-32, the Fab of MAb 166-32 was prepared by papain digestion using a commercial reagent kit (Pierce). The Fab was then tested for inhibitory activity on the alternative pathway hemolysis using unsensitized rabbit RBCs as described

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In order to study the cross-reactivity of MAb 166-32 with factor D from different animal species, alternative pathway hemolytic assays were performed using sera from different animal species. Fresh sera from different animal species (human, rhesus monkey, chimpanzee, baboon, cynomolgus monkey, sheep, dog, mouse, hamster, rat, rabbit, guinea pig and pig) were first tested for the CH50 values, which are defined as the dilution of the serum to achieve 50% lysis of unsensitized rabbit RBCs. The inhibitory activity of MAb 166-32 on the same hemolytic activity (CH50) of each serum was then tested and compared.

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To delineate the binding epitope on human factor D recognized by MAb 166-32, the reactivity of the antibody with human factor D on Western blots was first tested. MAb 166-32 did not react with SDS-denatured human factor D (either reduced or non-reduced) immobilized on nitrocellulose membrane. This result indicates the MAb 166-32 binds native but not denatured factor D.

Since MAb 166-32 does not inhibit the hemolytic activity of mouse and pig factor D as described in Example 7, it is likely that MAb 166-32 binds to a site on human factor D that has a high degree of difference in the amino acid sequence from those of mouse and pig factor D. Based on this concept, various factor D mutants and hybrids were made by replacing amino acid residues in human factor D with the corresponding amino acid residues in the pig counterpart, for mapping the binding epitope of Mab 166-32, as described below.

(1) Construction of factor D mutants and hybrids

Human factor D gene segments were obtained by polymerase chain reaction (PCR) using human adipocyte cDNA (Clontech, San Francisco, CA) as the template and appropriate oligonucleotide primers. Amplified DNA fragments were digested

temperature. After the wells were washed, peroxidase substrate solution was added for color development and OD450 was measured as described above. Purified thrombospondin (Calbiochem) was used as for a calibration standard in the assay.

IL-8 was measured by an ELISA kit (R & D Systems). IL-8, a proinflammatory cytokine, is produced by neutrophils, monocytes/macrophages, and T cells upon activation by anaphylatoxins.

The data from 5 paired circuits were statistically analyzed using 2-way ANOVA of factorial, randomized block design for all assays except hemolytic assays which used a paired T-test for analysis (see pg. 11 And Figure 26).. The data were represented as mean ± standard error.

(2) Results and conclusions

MAb 166-32 at 18 μg/ml inhibited completely the alternative complement activity measured by a hemolytic assay using unsensitized rabbit RBCs, whereas the negative control Mab G3-519 had no effect (Fig. 26). On the other hand, MAb 166-32 did not inhibit the classical pathway hemolysis of sensitized sheep RBCs. The selective inhibition of the alternative complement pathway is in agreement with the complete inhibition of the production of Bb (Fig. 27). Bb is the enzymatic product of factor B activation specific to the alternative complement pathway. On the other hand, there was an increase in the plasma concentration of C4d in both circuits treated with either MAb 166-32 or G3-519 (Fig. 28). C4d is a specific marker for activation of the classical complement pathway.

measurement of area at risk and infarct size, respectively. Coronary lymph and whole blood from the jugular vein were collected before ischemia and at the end of the experiment. These samples were used to measure the concentration of the injected antibodies and alternative pathway hemolytic activity.

The results show that the highest achievable concentration of MAb 166-32 in the coronary lymph was about 30 µg/ml, which is well below the concentration required for complete inhibition of dog factor D in the coronary circulation. The antibody was also detected in the systemic circulation, suggesting that the injected antibody dissipated outside of the heart. The data from the hemolytic assays show that alternative complement activity was not reduced; as is consistent with the fact that the concentration of the antibody was low. Therefore, it is not possible to draw a conclusion on the effect of MAb 166-32 in reperfusion from these experiments in dogs.

Example 14: Effects of MAb166-32 in Baboons Undergoing Cardiopulmonary Bypass

Anti-factor D MAb166-32 (murine antibody) was studied in a baboon model of hypothermic cardiopulmonary bypass (CPB) for pharmacokinetics and for its inhibitory effects on complement, neutrophils, monocytes, platelets, and tissue injury. Baboons was selected as the animal model because they have been extensively used as a non-human primate model in CPB studies (Hiramatsu Y et al., J. Lab. Clin. Med. 1997; 130: 412-420; Gikakis N et al., J.

Thorac. Cardiovasc. Surg. 1998; 116: 1043-1051) and MAb166-32 is equally effective at inhibiting baboon factor D and human factor D.

Fourteen healthy adult baboons (ca. 15 kg of body weight) were used in the study. The baboons were pre-screened for negative serological reactivity with MAb166-32 by ELISA. The baboons were assigned to two groups: 7 in the MAb166-32-treated group and 7 in a saline-control group. Baboons in the treatment group received a single intravenous bolus injection of MAb166-32 at 5 mg/kg, whereas baboons in the control group received an equal volume of saline. This dosage of MAb166-32 was chosen because it was estimated to be adequate to completely neutralize the factor D for about 7 hours, based on previously obtained data that MAb166-32 at 1 mg/kg completely inhibited factor D in rhesus monkeys for at least 1.5 hours. MAb166-32 inhibited baboon, monkey and human factor D equally well.

Baboons were sedated with an intramuscular injection of 10 mg/kg of ketamine hydrochloride, and anesthesia was induced with 5 mg/kg of thiopental sodium. The animals were then intubated, and general anesthesia was maintained with inhaled isoflurane.

Heparin (300 units/kg) was administered for anti-coagulation. After a median sternotomy was performed, the ascending aorta and the right atrium were cannulated with a 14-French aortic cannular (DLP, Inc. Grand Rapids, MI, USA) and a 26-French single-stage venous cannula (Polystan A/S, Varlose,

Denmark), respectively. Lactated Ringer's solution was then used to prime the extracorporeal circuit. The priming volume for the whole circuit was approximately 600 ml. This particular low-prime circuit was used to avoid the need for donor blood. During the CPB, the hematocrit was maintained at 26% to 28%. Pulmonary artery flow was assessed with a 12-mm perivascular flow probe (Transonic Systems, Ithaca, NY, USA), before and after CPB, for cardiac output measurements.

The CPB circuit consisted of a conventional non-pulsatile roller pump (Stockert, Irvine, CA, USA), a hollow-fiber membrane oxygenator (Capiorx SX10; Terumo Corp., Tokyo, Japan), an arterial filter (Terumo Corp.), and silicone elastomer tubing (Dow Corning, Inc., Midland, MI, USA). The pump flow rate was maintained at 80 ml/kg/min. During CPB, the mean arterial pressure was maintained at 50 – 60 mm Hg by adding isoflurane through the oxygenator inflow conduit. At the end of CPB, protamine (1 mg/100 units of heparin) was administered for heparin neutralization. The heart rate, systemic arterial pressure, central venous pressure, and pulmonary arterial pressure was continuously monitored. At the end of the experiment, each animal was euthanized with intravenous boluses of Beuthanasia-D (0.22 mg/kg).

Blood samples were taken from the animals for assays at different time points: Before injection of MAb166-32 or saline (at 0 hour), after injection of MAb166-32 or saline (at 45 minutes), before CPB (at 1 hour), 10 minutes in CPB

at 37°C, 25 minutes in CPB at 27°C, 85 minutes in CPB at 27°C, 105 minutes in CPB at 37°C after re-warming, 135 minutes in CPB at 37°C prior to protamine administration (at 3.25 hours), 30 minutes after CPB at 37°C, 1 hour after CPB at 37°C, 2 hours after CPB at 37°C, 6 hours after CPB at 37°C (at 9.25 hours), and finally 18 hours after CPB at 37°C (at 21.25 hours). Experiments with 8 of the 14 baboons (4 in the treatment group and 4 in the control group) were terminated at 6 hours after CPB, whereas the remaining animals (3 in the treatment group and 3 in the control group) were terminated 18 hours after CPB.

Plasma and whole blood samples were used in different assays to measure:

(1) Plasma concentration of free MAb166-32 by ELISA using factor D as the coating antigen;

(2) Functional activity of factor D in baboon plasma as determined by two hemolytic assays: rabbit red blood cells for the alternative complement pathway and sensitized chicken red blood cells for the classical complement pathway ;

(3) Plasma concentration of complement Bb, C4d, and C3a by ELISA (Quidel Corp., San Diego, CA, USA);

(4) Expression level of CD11b on neutrophils and monocytes by immunofluorocytometric methods;

(5) Expression level of CD62P on platelets by immunofluorocytometric methods;

(6) Plasma concentration of IL-6 by ELISA (BioSource International, Inc., Camarillo, CA, USA); and

(7) Plasma concentration of lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase MB isoenzymes (CK-MB), and creatinine.

The data were analyzed statistically by student's T-test (for C4d) and 2-way ANOVA of repeated measurements (for other parameters) (significant at $p<0.05$). The data were represented as mean±SEM.

(II) Results and Discussion

In this study, baboons were treated with a single intravenous bolus injection of 5 mg/kg of MAb166-32. The plasma concentrations of the free antibody were measured by an ELISA using human factor D as coating antigen (Fig. 39). At 45 minutes after the antibody injection, the plasma concentration of free MAb166-32 was 68.3±9.9 μg/ml. The antibody concentration then decreased to 23.4±4.4 μg/ml at 10 minutes after CPB, as a result of hemodilution upon the initiation of CPB. The antibody concentration remained at 10 –13 μg/ml until 3 hours after CPB. The antibody concentration reduced to 6.2±2.3 μg/ml at 6 hours after CPB and to 1.7±3.9 μg/ml at 18 hours after CPB.

Using an alternative complement hemolytic assay with rabbit red blood cells, the functional activity of factor D in the plasma samples from the MAb166-32 treated animals was measured (Fig. 40). The alternative complement hemolytic activity of the baboon plasma samples was completely inhibited at 6

hours after CPB. At 18 hours after CPB, the inhibition was reduced to 79.3±10%. The data are consistent with the presence of free MAb166-32 in the circulation until 18 hours after CPB (Fig. 39). In hemolytic assays using sensitized chicken red blood cells to measure the classical complement activity, the corresponding plasma samples from the MAb166-32 treated animals did not show any reduction in the classical complement activity by the antibody (Fig. 40). These results confirm that MAb166-32 is a specific inhibitor of the alternative complement pathway.

The specificity of MAb166-32 in inhibiting the alternative complement pathway was also demonstrated by the complete inhibition of Bb formation (Fig. 41). Bb is the activation product of factor B upon proteolytic cleavage by factor D. The increase in Bb formation in the control animals is attributed to the activation of the alternative complement pathway during CPB. The reduction of plasma Bb concentration below the baseline in the MAb166-32 treated animals could be due to the inhibition of the physiological activation of the alternative complement in the animals.

Activation of the classical complement pathway was determined by measuring C4d, which is a specific marker for the activation of C4 in the classical complement pathway. In the study, the plasma levels of C4d in both the MAb166-32 treated and control animals were relatively stable with reference to the baseline (Fig. 42). However, there was an increase of C4d in both animal

groups after neutralization of heparin with protamine at the end of the CPB, indicating the activation of the classical pathway as reported earlier in other studies (Kirklin JK et al., Ann. Thorac. Surg. 1986; 41: 193-199; Carr JA et al., J. Cardiovasc. Surg. (Torino) 1999; 40: 659-666).

Activation of complement is shown by an increase in plasma C3a in the control animals (Fig. 43). In contrast, animals treated with MAb166-32 show almost complete inhibition of C3a production. A slight increase of C3a level is observed in the MAb166-32 treated animals after neutralization of heparin with protamine. Together, the results from Figs. 41, 42 and 43 support the notion that complement activation during CPB is predominantly via the alternative complement pathway. Due to the lack of cross-reactivity of the reagents in the commercial ELISA kits for baboon C5a and sC5b-9 (Quidel and Becton Dickinson, respectively), their concentrations were not determined.

Activation of neutrophils and monocytes in the baboons was examined by measuring CD11b (α-integrin) expression using immunofluorcytometric methods. In the control animals, the CD11b expression on neutrophils increased rapidly and reached the maximum at about 85 minutes after the start of CPB (209±42.9% of the baseline) (Fig. 44). It then declined slowly back to around the baseline. In contrast, the increase of CD11b expression on neutrophils in the MAb166-32 treated animals was delayed and smaller in magnitude (Fig. 44). The maximum level of CD11b expression was 129.3%±5.5% of the baseline.

Inhibition of the increase in CD11b expression on monocytes from the MAb166-32 treated animals was also observed (Fig. 45).

Activation of platelets in the baboons was examined by measuring CD62P (P-selectin) expression using immunofluorocytometric methods. Both the control and MAb166-32 treated animals show a similar pattern of inhibition of CD62P expression (Fig. 46). The exact cause of the inhibition of CD62P expression is unclear.

The effect of MAb166-32 treatment on pro-inflammatory cytokines was also examined. Baboons treated with MAb166-32 had a significantly smaller increase in plasma IL-6 concentration as compared to the control animals (Fig. 47).

The effects of MAb166-32 treatment on tissue injury of various organs were also studied. The increase of plasma LDH levels was significantly reduced in the MAb166-32 treated animals at 3 and 6 hours after CPB (Fig. 48). This indicates protection against tissue injury. Specifically for myocardial injury, the increase of both plasma CK and CK-MB levels at 6 and 18 hours after CPB was significantly lower in the MAb166-32 treated animals as compared to the control animals (Figs. 49 and 50).

The renal function of the baboons was also examined. A reduction in the increase of plasma creatinine was found in the baboons treated with MAb166-32 as compared to the control animals at 18 hours after CPB (Fig. 52).

As for pulmonary functions, the dynamic lung compliance of the MAb166-32 treated animals was higher than that of the control animals during the early phase of the open-chest surgery (Fig. 51). However, there was no significant difference in the lung compliance between the two groups of animals during and after CPB. The initial increase of the dynamic lung compliance in the control animals is probably attributed to the open-chest procedure. The higher dynamic lung compliance in the MAb166-32 treated animals could be due to the protection against surgical trauma which was shown to be associated with complement activation via the alternative complement pathway. (Gu YJ et al., Chest 1999; 116: 892-898).

In conclusion, anti-factor D MAb166-32 is effective in inhibiting the activation of the alternative complement pathway in a baboon model of CPB. Inhibition of the alternative complement pathway by MAb166-32 effectively reduces the activation of neutrophils and monocytes, as well as the production of IL6. Treatment with MAb166-32 confers protection against myocardial and renal injury. The alternative complement pathway may play a predominant role in the inflammation and tissue injury caused by extracorporeal circulation, surgical trauma and ischemia/reperfusion. Therefore MAb166-32 could be potentially useful for the treatment of systemic inflammatory response syndromes in CPB.

The foregoing description, terms, expressions and examples are exemplary only and not limiting. The invention includes all equivalents of the foregoing

embodiments, both known and unknown. The invention is limited only by the claims which follow and not by any statement in any other portion of this document or in any other source.

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(1) Construction of factor D mutants and hybrids

Human factor D gene segments were obtained by polymerase chain reaction (PCR) using human adipocyte cDNA (Clontech, San Francisco, CA) as the template

temperature. After the wells were washed, peroxidase substrate solution was added for color development and OD450 was measured as described above. Purified thrombospondin (Calbiochem) was used as for a calibration standard in the assay.

IL-8 was measured by an ELISA kit (R & D Systems). IL-8, a proinflammatory cytokine, is produced by neutrophils, monocytes/macrophages, and T cells upon activation by anaphylatoxins.

The data from 5 paired circuits were statistically analyzed using 2-way ANOVA of factorial, randomized block design for all assays except hemolytic assays which used a paired T-test for analysis (see pg. 11 And Figure 26).. The data were represented as mean ± standard error.

(2) Results and conclusions

MAb 166-32 at 18 μg/ml inhibited completely the alternative complement activity measured by a hemolytic assay using unsensitized rabbit RBCs, whereas the negative control Mab G3-519 had no effect (Fig. 26). On the other hand, MAb 166-32 did not inhibit the classical pathway hemolysis of sensitized sheep RBCs. The selective inhibition of the alternative complement pathway is in agreement with the complete inhibition of the production of Bb (Fig. 27). Bb is the enzymatic product of factor B activation specific to the alternative complement pathway. On the other hand, there was an increase in the plasma concentration of C4d in both circuits treated with either MAb 166-32 or G3-519 (Fig. 28). C4d is a specific marker for activation of the classical complement pathway.

measurement of area at risk and infarct size, respectively. Coronary lymph and whole blood from the jugular vein were collected before ischemia and at the end of the experiment. These samples were used to measure the concentration of the injected antibodies and alternative pathway hemolytic activity.

The results show that the highest achievable concentration of MAb 166-32 in the coronary lymph was about 30 µg/ml, which is well below the concentration required for complete inhibition of dog factor D in the coronary circulation. The antibody was also detected in the systemic circulation, suggesting that the injected antibody dissipated outside of the heart. The data from the hemolytic assays show that alternative complement activity was not reduced; as is consistent with the fact that the concentration of the antibody was low. Therefore, it is not possible to draw a conclusion on the effect of MAb 166-32 in reperfusion from these experiments in dogs.

Example 14: Effects of [Mab] MAb 166-32 in Baboons Undergoing Cardiopulmonary Bypass

Anti-factor D [Mab] MAb 166-32 (murine antibody) was studied in a baboon model of hypothermic cardiopulmonary bypass (CPB) for pharmacokinetics and for its inhibitory effects on complement, neutrophils, monocytes, platelets, and tissue injury. Baboons was selected as the animal model because they have been extensively used as a non-human primate model in CPB studies (Hiramatsu Y et al., J. Lab. Clin. Med. 1997; 130: 412-420;

Gikakis N et al., J. Thorac. Cardiovasc. Surg. 1998; 116: 1043-1051) and [Mab] MAb 166-32 is equally effective at inhibiting baboon factor D and human factor D.

Fourteen healthy adult baboons (ca. 15 kg of body weight) were used in the study. The baboons were pre-screened for negative serological reactivity with [Mab] MAb 166-32 by ELISA. The baboons were assigned to two groups: 7 in the [Mab] MAb 166-32-treated group and 7 in a saline-control group. Baboons in the treatment group received a single intravenous bolus injection of [Mab] MAb 166-32 at 5 mg/kg, whereas baboons in the control group received an equal volume of saline. This dosage of [Mab] MAb 166-32 was chosen because it was estimated to be adequate to completely neutralize the factor D for about 7 hours, based on previously obtained data that [Mab] MAb 166-32 at 1 mg/kg completely inhibited factor D in rhesus monkeys for at least 1.5 hours. [Mab] MAb 166-32 inhibited baboon, monkey and human factor D equally well.

Baboons were sedated with an intramuscular injection of 10 mg/kg of ketamine hydrochloride, and anesthesia was induced with 5 mg/kg of thiopental sodium. The animals were then intubated, and general anesthesia was maintained with inhaled isoflurane.

Heparin (300 units/kg) was administered for anti-coagulation. After a median sternotomy was performed, the ascending aorta and the right atrium were cannulated with a 14-French aortic cannular (DLP, Inc. Grand Rapids, MI,

USA) and a 26-French single-stage venous cannula (Polystan A/S, Varlose, Denmark), respectively. Lactated Ringer's solution was then used to prime the extracorporeal circuit. The priming volume for the whole circuit was approximately 600 ml. This particular low-prime circuit was used to avoid the need for donor blood. During the CPB, the hematocrit was maintained at 26% to 28%. Pulmonary artery flow was assessed with a 12-mm perivascular flow probe (Transonic Systems, Ithaca, NY, USA), before and after CPB, for cardiac output measurements.

The CPB circuit consisted of a conventional non-pulsatile roller pump (Stockert, Irvine, CA, USA), a hollow-fiber membrane oxygenator (Capiorx SX10; Terumo Corp., Tokyo, Japan), an arterial filter (Terumo Corp.), and silicone elastomer tubing (Dow Corning, Inc., Midland, MI, USA). The pump flow rate was maintained at 80 ml/kg/min. During CPB, the mean arterial pressure was maintained at 50 – 60 mm Hg by adding isoflurane through the oxygenator inflow conduit. At the end of CPB, protamine (1 mg/100 units of heparin) was administered for heparin neutralization. The heart rate, systemic arterial pressure, central venous pressure, and pulmonary arterial pressure was continuously monitored. At the end of the experiment, each animal was euthanized with intravenous boluses of Beuthanasia-D (0.22 mg/kg).

Blood samples were taken from the animals for assays at different time points: Before injection of [Mab] MAb 166-32 or saline (at 0 hour), after injection

of [Mab] MAb 166-32 or saline (at 45 minutes), before CPB (at 1 hour), 10 minutes in CPB at 37°C, 25 minutes in CPB at 27°C, 85 minutes in CPB at 27°C, 105 minutes in CPB at 37°C after re-warming, 135 minutes in CPB at 37°C prior to protamine administration (at 3.25 hours), 30 minutes after CPB at 37°C, 1 hour after CPB at 37°C, 2 hours after CPB at 37°C, 6 hours after CPB at 37°C (at 9.25 hours), and finally 18 hours after CPB at 37°C (at 21.25 hours). Experiments with 8 of the 14 baboons (4 in the treatment group and 4 in the control group) were terminated at 6 hours after CPB, whereas the remaining animals (3 in the treatment group and 3 in the control group) were terminated 18 hours after CPB.

Plasma and whole blood samples were used in different assays to measure:

(1) Plasma concentration of free [Mab] MAb 166-32 by ELISA using factor D as the coating antigen;

(2) Functional activity of factor D in baboon plasma as determined by two hemolytic assays: rabbit red blood cells for the alternative complement pathway and sensitized chicken red blood cells for the classical complement pathway ;

(3) Plasma concentration of complement Bb, C4d, and C3a by ELISA (Quidel Corp., San Diego, CA, USA);

(4) Expression level of CD11b on neutrophils and monocytes by immunofluorocytometric methods;

(5) Expression level of CD62P on platelets by immunofluorocytometric methods;

(6) Plasma concentration of IL-6 by ELISA (BioSource International, Inc., Camarillo, CA, USA); and

(7) Plasma concentration of lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase MB isoenzymes (CK-MB), and creatinine.

The data were analyzed statistically by student's T-test (for C4d) and 2-way ANOVA of repeated measurements (for other parameters) (significant at $p<0.05$). The data were represented as mean±SEM.

(II) Results and Discussion

In this study, baboons were treated with a single intravenous bolus injection of 5 mg/kg of [Mab] MAb 166-32. The plasma concentrations of the free antibody were measured by an ELISA using human factor D as coating antigen (Fig. 39). At 45 minutes after the antibody injection, the plasma concentration of free [Mab] MAb 166-32 was 68.3±9.9 μg/ml. The antibody concentration then decreased to 23.4±4.4 μg/ml at 10 minutes after CPB, as a result of hemodilution upon the initiation of CPB. The antibody concentration remained at 10 –13 μg/ml until 3 hours after CPB. The antibody concentration reduced to 6.2±2.3 μg/ml at 6 hours after CPB and to 1.7±3.9 μg/ml at 18 hours after CPB.

Using an alternative complement hemolytic assay with rabbit red blood cells, the functional activity of factor D in the plasma samples from the [Mab] MAb 166-32 treated animals was measured (Fig. 40). The alternative

complement hemolytic activity of the baboon plasma samples was completely inhibited at 6 hours after CPB. At 18 hours after CPB, the inhibition was reduced to 79.3±10%. The data are consistent with the presence of free [Mab] MAb 166-32 in the circulation until 18 hours after CPB (Fig. 39). In hemolytic assays using sensitized chicken red blood cells to measure the classical complement activity, the corresponding plasma samples from the [Mab] MAb 166-32 treated animals did not show any reduction in the classical complement activity by the antibody (Fig. 40). These results confirm that [Mab] MAb 166-32 is a specific inhibitor of the alternative complement pathway.

The specificity of [Mab] MAb 166-32 in inhibiting the alternative complement pathway was also demonstrated by the complete inhibition of Bb formation (Fig. 41). Bb is the activation product of factor B upon proteolytic cleavage by factor D. The increase in Bb formation in the control animals is attributed to the activation of the alternative complement pathway during CPB. The reduction of plasma Bb concentration below the baseline in the [Mab] MAb 166-32 treated animals could be due to the inhibition of the physiological activation of the alternative complement in the animals.

Activation of the classical complement pathway was determined by measuring C4d, which is a specific marker for the activation of C4 in the classical complement pathway. In the study, the plasma levels of C4d in both the [Mab] MAb 166-32 treated and control animals were relatively stable with reference to

the baseline (Fig. 42). However, there was an increase of C4d in both animal groups after neutralization of heparin with protamine at the end of the CPB, indicating the activation of the classical pathway as reported earlier in other studies (Kirklin JK et al., Ann. Thorac. Surg. 1986; 41: 193-199; Carr JA et al., J. Cardiovasc. Surg. (Torino) 1999; 40: 659-666).

Activation of complement is shown by an increase in plasma C3a in the control animals (Fig. 43). In contrast, animals treated with [Mab] MAb 166-32 show almost complete inhibition of C3a production. A slight increase of C3a level is observed in the [Mab] MAb 166-32 treated animals after neutralization of heparin with protamine. Together, the results from Figs. 41, 42 and 43 support the notion that complement activation during CPB is predominantly via the alternative complement pathway. Due to the lack of cross-reactivity of the reagents in the commercial ELISA kits for baboon C5a and sC5b-9 (Quidel and Becton Dickinson, respectively), their concentrations were not determined.

Activation of neutrophils and monocytes in the baboons was examined by measuring CD11b (α-integrin) expression using immunofluorcytometric methods. In the control animals, the CD11b expression on neutrophils increased rapidly and reached the maximum at about 85 minutes after the start of CPB (209±42.9% of the baseline) (Fig. 44). It then declined slowly back to around the baseline. In contrast, the increase of CD11b expression on neutrophils in the [Mab] MAb 166-32 treated animals was delayed and smaller in magnitude (Fig.

44). The maximum level of CD11b expression was 129.3%±5.5% of the baseline. Inhibition of the increase in CD11b expression on monocytes from the [Mab] MAb 166-32 treated animals was also observed (Fig. 45).

Activation of platelets in the baboons was examined by measuring CD62P (P-selectin) expression using immunofluorocytometric methods. Both the control and [Mab] MAb 166-32 treated animals show a similar pattern of inhibition of CD62P expression (Fig. 46). The exact cause of the inhibition of CD62P expression is unclear.

The effect of [Mab] MAb 166-32 treatment on pro-inflammatory cytokines was also examined. Baboons treated with [Mab] MAb 166-32 had a significantly smaller increase in plasma IL-6 concentration as compared to the control animals (Fig. 47).

The effects of [Mab] MAb 166-32 treatment on tissue injury of various organs were also studied. The increase of plasma LDH levels was significantly reduced in the [Mab] MAb 166-32 treated animals at 3 and 6 hours after CPB (Fig. 48). This indicates protection against tissue injury. Specifically for myocardial injury, the increase of both plasma CK and CK-MB levels at 6 and 18 hours after CPB was significantly lower in the [Mab] MAb 166-32 treated animals as compared to the control animals (Figs. 49 and 50).

The renal function of the babcons was also examined. A reduction in the increase of plasma creatinine was found in the baboons treated with [Mab] MAb 166-32 as compared to the control animals at 18 hours after CPB (Fig. 52).

As for pulmonary functions, the dynamic lung compliance of the [Mab] MAb 166-32 treated animals was higher than that of the control animals during the early phase of the open-chest surgery (Fig. 51). However, there was no significant difference in the lung compliance between the two groups of animals during and after CPB. The initial increase of the dynamic lung compliance in the control animals is probably attributed to the open-chest procedure. The higher dynamic lung compliance in the [Mab] MAb 166-32 treated animals could be due to the protection against surgical trauma which was shown to be associated with complement activation via the alternative complement pathway. (Gu YJ et al., Chest 1999; 116: 892-898).

The renal function of the baboons was also examined. A reduction in the increase of plasma creatinine was found in the baboons treated with [Mab] MAb 166-32 as compared to the control animals at 18 hours after CPB (Fig. 52).

In conclusion, anti-factor D [Mab] MAb 166-32 is effective in inhibiting the activation of the alternative complement pathway in a baboon model of CPB. Inhibition of the alternative complement pathway by [Mab] MAb 166-32 effectively reduces the activation of neutrophils and monocytes, as well as the production of IL6. Treatment with [Mab] MAb 166-32 confers protection against

myocardial and renal injury. The alternative complement pathway may play a predominant role in the inflammation and tissue injury caused by extracorporeal circulation, surgical trauma and ischemia/reperfusion. Therefore [Mab] MAb 166-32 could be potentially useful for the treatment of systemic inflammatory response syndromes in CPB.

The foregoing description, terms, expressions and examples are exemplary only and not limiting. The invention includes all equivalents of the foregoing embodiments, both known and unknown. The invention is limited only by the claims which follow and not by any statement in any other portion of this document or in any other source.